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During anaphase of mitosis, sister chromatids are separated by the dynamic mitotic spindle. Failure to equally distribute the chromosomal DNA can lead to genetic loss and aneuploidy. In eukaryotic cells, the spindle assembly checkpoint monitors the attachments between chromatids and the spindle to initiate a cell cycle delay if the attachments are defective. The fission yeast, *Schizosaccharomyces pombe*, is a useful system for discovering and characterizing components of this regulatory pathway because genetic approaches can be coupled with excellent cytology. Previous work in the Sazer laboratory identified the first two spindle checkpoint genes in *S. pombe*: *mad2* and *mph1*. Cells in which the *mph1* or *mad2* gene is deleted are sensitive to the microtubule inhibitor, thiabendazole (TBZ), because they fail to arrest at metaphase to repair the spindle, and therefore undergo a lethal division. Overexpression of either *mad2* or *mph1* activates the spindle checkpoint, arrests cells prior to anaphase, and is toxic to wild type cells. *mph1* acts upstream of *mad2*, and the toxic effect of *mph1* overexpression is suppressed by a *mad2* deletion.

This project aims to discover and characterize novel spindle checkpoint genes as suppressors of *mph1* overexpression. The *mph1* overexpression screen has yielded mutations in three known spindle checkpoint genes and a mutation, called 4101, in an apparently novel component of the spindle checkpoint pathway. 4101 mutant cells are checkpoint deficient as indicated by their failure to arrest the cell cycle in response to spindle disruption. 4101 cells also exhibit temperature sensitive lethality, and mitotic defects are observed at the restrictive temperature in the absence of TBZ. Plasmid rescue of the temperature sensitive lethality will be exploited to identify the gene mutated in strain 4101. The relationship between the gene mutated in strain 4101 and known components of the spindle checkpoint pathway will be determined using classical and molecular genetics.

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INTRODUCTION

For cells to proliferate, they must completely duplicate their genetic material during S phase of the cell cycle and then partition one complete set of chromosomes into each of the daughter cells during anaphase. Failure to achieve equal segregation of the chromosomal DNA can lead to genetic loss, lethality, or aneuploidy. This aneuploidy is a feature of cancer cells which typically correlates with aggressive tumors and a poor prognosis (Hartwell and Kastan, 1994). Genetic loss and aneuploidy occur rarely because eukaryotic cells have checkpoints to monitor such critical events in the cell cycle and ensure that downstream events can not proceed until the successful completion of upstream events (Hartwell and Weinert, 1989). The spindle assembly checkpoint system ensures that chromosome segregation is not initiated before the chromosomes are attached to a properly assembled bipolar mitotic spindle (Wells and Murray, 1996). When defects are detected, the checkpoint system blocks the cell cycle at metaphase, the stage of mitosis in which the sister chromatids align at the equatorial plate. This delay allows for spindle repair, thus preventing the gain or loss of chromosomes in the daughter cells. Cells mutated in spindle checkpoint genes fail to arrest the cell cycle in response to spindle damage because they fail to detect the defect or because they can not restrain the cell cycle. These cells undergo an aberrant division that results in mis-segregation of DNA and loss of genetic information (Hardwick, 1998). Loss of the spindle checkpoint may result in the multiple genetic changes that mark the development of tumor cells (Cahill et al., 1998; Lengauer et al., 1998; Orr-Weaver and Weinberg, 1998). In support of this prediction, loss of checkpoint function has been observed in many human cancer cell lines (Cahill et al., 1998; Takahashi et al., 1999), and decreased expression levels of the checkpoint component, *hsMAD2*, have been demonstrated in human breast cancers (Li and Benezra, 1996) and shown to promote lung tumors in mice (Michel et al., 2001).

The first spindle checkpoint genes were identified by two independent screens in the budding yeast, *Saccharomyces cerevisiae*(Hoyt et al., 1991; Li and Murray, 1991). These MAD and BUB screens identified six spindle checkpoint mutants by their sensitivity to microtubule destabilizing drugs. The *mad* and *bub* mutants are sensitive to microtubule disruption because they fail to arrest the cell cycle in response to this damage, resulting in mis-segregation of chromosomes and loss of genetic material. Genes that encode mammalian homologues of several of these yeast checkpoint genes, such as *hsMAD2*, have been identified based on sequence similarity to the yeast proteins (Cahill et al., 1999; Fisk and Winey, 2001; Jin et al., 1998; Li and Benezra, 1996; Ouyang et al., 1998; Taylor et al., 1998). The fission yeast, *Schizosaccharomyces pombe*, is a useful system for discovering new components of the spindle checkpoint pathway because genetic approaches can be coupled with excellent cytology. Previous work in the Sazer laboratory identified the first two spindle checkpoint genes in *S. pombe*: *mad2* and *mph1* (He et al., 1998; He et al., 1997). Overexpression of either *mad2* or *mph1* activates the spindle checkpoint, arrests cells prior to anaphase, and is toxic to wild type cells. In the *S. pombe* spindle checkpoint pathway, *mph1* acts upstream of *mad2*. Therefore, while *mph1* overexpression prevents cell cycle progression in wild type cells, this toxic effect is not observed in *mad2* deletion mutants which lack a functional checkpoint. Based on this observation, a genetic screen was designed to identify new components of the spindle checkpoint pathway as suppressors of *mph1* overexpression toxicity. It is predicted that these new spindle checkpoint components will have mammalian homologues, and characterization of the *S. pombe* proteins will provide a better understanding of the spindle checkpoint pathway and may reveal new information about the process of tumorigenesis.

BODY

Background

Previous work in the Sazer laboratory identified the first two spindle checkpoint genes in *S. pombe*: *mad2* and *mph1* (He et al., 1998; He et al., 1997). Overexpression of either *mad2* or *mph1* activates the spindle checkpoint and arrests cells prior to anaphase. Therefore, overexpressing *mad2* or *mph1* is toxic to cells because it causes them to arrest the cell cycle . In the spindle checkpoint pathway, *mph1* acts upstream of *mad2*, and the toxic effect of *mph1* overexpression is suppressed by a *mad2* deletion. This observation was employed in a genetic screen to identify new components of the spindle checkpoint pathway as suppressors of *mph1* overexpression toxicity.

The aim of this project is to identify and characterize components of the spindle checkpoint pathway in *S. pombe*. The following sections detail the current results of this project. Figure 1 describes the strategy I have employed: (1) to determine if the *mph1* overexpression screen was successful in identifying spindle checkpoint mutants, (2) to determine if the screen has identified novel spindle checkpoint genes, and (3) to identify the gene mutated in selected strains.

The *mph1* overexpression screen

The *mph1* overexpression screen was performed by Xiangwei He, a former graduate student in the Sazer laboratory. Fission yeast cells expressing *mph1* from a regulatable promoter were randomly mutagenized as described in Figure 1. Approximately ten million cells were screened to identify mutagenized strains which can survive *mph1* overexpression. The initial test used to determine if these suppressors of *mph1* overexpression are spindle checkpoint mutants utilized the microtubule destabilizing drug, thiabendazole (TBZ). All spindle checkpoint mutants are sensitive to TBZ because they fail to arrest the cell cycle in response to microtubule disruption, resulting in chromosome mis-segregation followed by genetic loss and cell death. Therefore, the suppressor strains from the screen were tested for sensitivity to TBZ. 185 strains were isolated that survived *mph1* overexpression and were sensitive to TBZ, 55 of which were highly sensitive, indicating that these strains are likely to contain mutations in spindle checkpoint genes.

The strains from the *mph1* overexpression screen were tested for temperature sensitive lethality (ts) to determine if the screen may have identified genes that are essential for spindle checkpoint function as well as viability. Most previously characterized spindle checkpoint mutants are not essential for growth because the spindle checkpoint is required only when the chromosomes are not properly attached to a functional bipolar mitotic spindle. However, some spindle checkpoint genes are essential because they have multiple functions, one of which is required for cell division. Two essential spindle checkpoint genes in *S. cerevisiae* are *mps1* (Weiss and Winey, 1996) and *ndc10* (Goh and Kilmartin, 1993; Tavormina and Burke, 1998). The *S. cerevisiae mps1* gene encodes a spindle checkpoint protein that also functions in duplication of the spindle pole body, an event that is critical for the formation of a bipolar spindle. The *ndc10* gene encodes a checkpoint protein that is also required for the formation of the kinetochore, a chromosomal DNA and protein structure that mediates attachment between chromosomes and the mitotic spindle. The *mph1* overexpression screen has identified 107 suppressor strains that are ts. To date, I have focused on three of these strains which are TBZ-sensitive as well as ts. Perhaps these strains will identify new *S. pombe* spindle checkpoint genes that are essential for viability.

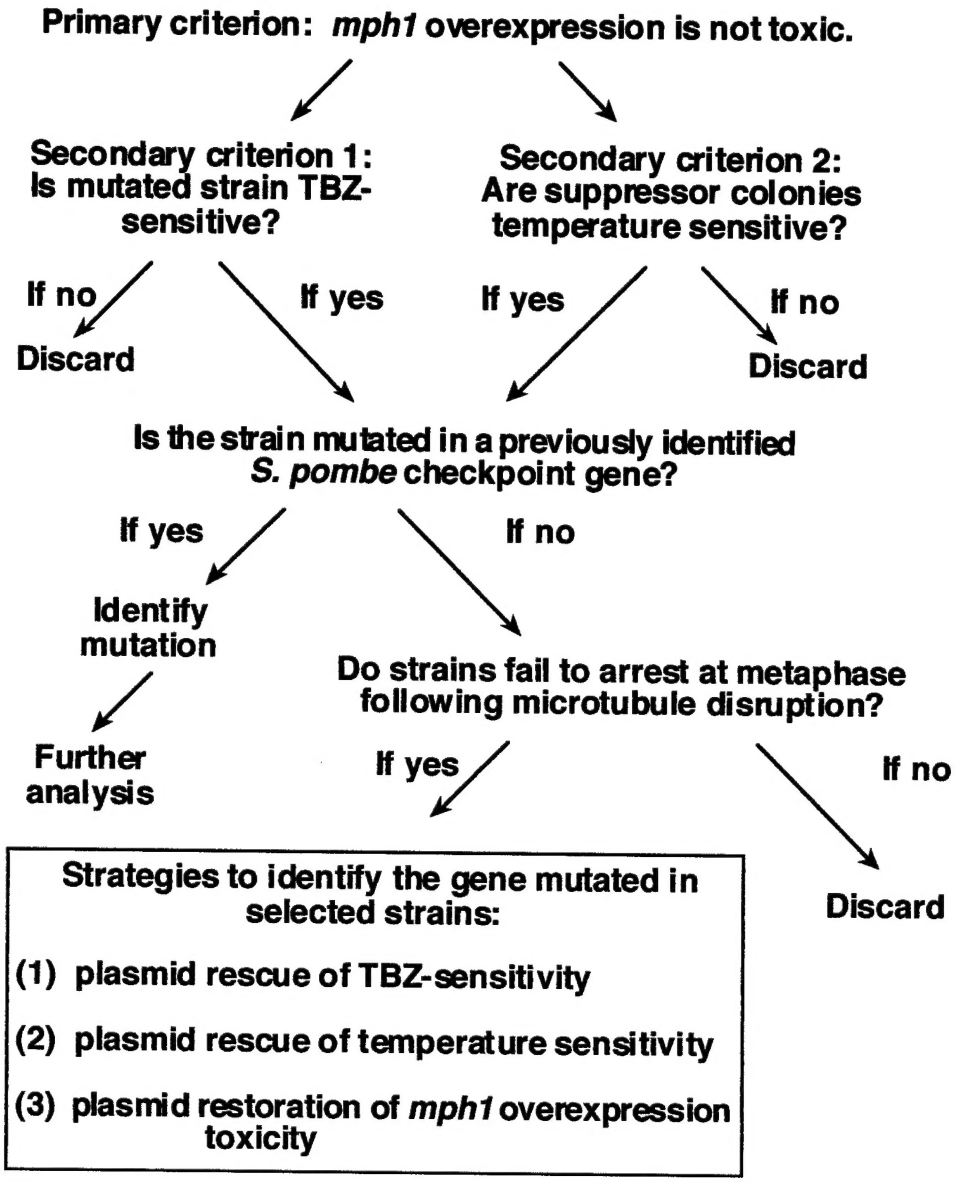

Figure 1: Description of the genetic screen and cloning strategy to identify new spindle checkpoint genes. Wild type cells, in which *mph1* is expressed from a thiamine repressible *nmt1* promoter (Maundrell, 1993), were mutagenized with nitrosoguanidine and plated onto EMM lacking thiamine to induce *mph1* overexpression. Viable mutant cells were recovered and tested for sensitivity to microtubule destabilization by streaking to 15 μg/mL TBZ at 29°C. The suppressors were tested for temperature sensitivity by replica plating to EMM with the vital dye, phloxine B, at 36°C. Mutant strains were crossed to deletions of known spindle checkpoint genes to determine if the screen identified known spindle checkpoint components. To determine which strains fail to arrest at metaphase in response to spindle defects, microtubules were disrupted by treatment with TBZ, treatment with carbendazim (CBZ), or the *cut7*^{ts} kinesin motor mutation (Hagan and Yanagida, 1990). Flow cytometry was used to determine if cells arrested the cell cycle in response to spindle disruption (see Figure 2).

Specific Aim 1: Identify and characterize additional components of the spindle checkpoint pathway in *S. pombe*.

(1) The *mph1* overexpression screen has identified three known spindle checkpoint genes.

TBZ-Sensitive Strains

To begin characterizing the strains from the *mph1* overexpression screen, 17 highly TBZ-sensitive mutants that grow normally in the absence of TBZ were analyzed. These strains were subjected to linkage analysis to determine if they are likely to be mutated in a known spindle checkpoint gene. Linkage analysis was performed using null mutations of the known spindle checkpoint genes in *S. pombe*: *mad1*, *mad2*, *mad3*, *bub1*, and *bub3* (Bernard et al., 1998; He et al., 1997)(Hardwick, personal communication).

Four of the strains from the *mph1* overexpression screen contain a mutation that is tightly linked to a known spindle checkpoint gene. Strain 1207 and strain 1221 contain mutations in a gene tightly linked to *bub1*. A plasmid carrying the *bub1* gene was able to confer TBZ resistance to strain 1207 and strain 1221, a confirmation that these strains are mutated in the *bub1* gene. Sequencing the *bub1* gene in strain 1207 revealed a mutation that would result in an amino acid change at position 988, which would cause a premature truncation of the protein in the highly conserved protein kinase domain.

Strain 1208 contains a mutation tightly linked to the *bub3* gene. To confirm that strain 1208 is mutated in the *bub3* gene, I have shown that the 1208 mutation is unable to complement a *bub3* null mutation. Finally, strain 1201 contains a mutation that is tightly linked to *mad1*. These results indicate that the *mph1* overexpression screen has successfully identified three known spindle checkpoint genes, validating the strategy of the screen and making it likely that the screen will identify novel genes in this pathway. I am currently focusing on 10 of the 17 strains subjected to linkage analysis because they contain only one mutation that confers the TBZ-sensitive phenotype. Six of these strains do not contain a mutation in a known spindle checkpoint component.

Novel mutations in the known spindle checkpoint genes will provide new tools to further our understanding of how the spindle checkpoint is activated and how it initiates the cell cycle arrest. The *bub1* gene in strain 1207 is mutated in the highly conserved kinase domain, which appears to be necessary for checkpoint activation in budding yeast (Roberts et al., 1994). It is predicted that this mutant *bub1* allele would encode a truncated bub1 protein that deletes 56 amino acids from the carboxy terminus. Preliminary characterization indicates that the phenotype of the 1207 mutant *bub1* strain is different from the phenotype of the *bub1* deletion, suggesting that strain 1207 may be useful in experiments addressing the importance of the Bub1p amino terminus in checkpoint function.

Temperature Sensitive Strains

To begin characterizing the ts strains from the *mph1* overexpression screen, 29 mutants were analyzed. Seven strains were set aside due to weak phenotypes or growth problems that would limit the ability to perform genetic experiments. Like the *S. cerevisiae MAD* and *BUB* genes, spindle checkpoint genes in *S. pombe* are not essential for viability (Bernard et al., 1998; He et al., 1997; Hoyt et al., 1991; Li and Murray, 1991). However, it was possible but not likely that a ts mutant strain could contain a mutation in a known spindle checkpoint gene that is not required for viability if the mutant protein can interfere with growth, thus conferring a unique phenotype that is not observed in the deletion mutant. Therefore, nine ts strains were subjected to the linkage analysis described above using deletions of the following spindle checkpoint genes in *S. pombe*: *mad2*, *mad3*, *bub1*, and *bub3*. Tight linkage was not observed between the ts mutations and any of these known spindle checkpoint

genes, indicating that the ts strains may identify a novel class of spindle checkpoint mutants in *S. pombe*.

(2) The *mph1* overexpression screen has identified one novel component of the spindle checkpoint pathway.

By definition, spindle checkpoint mutants are unable to arrest the cell cycle at metaphase when the attachment between the spindle microtubules and the kinetochores is compromised. This definition was utilized to employ additional assays to determine if the strains from the *mph1* overexpression screen are definitively checkpoint deficient. These additional assays are necessary for two reasons. First, it is possible that some of the *mph1* overexpression suppressor strains survived the screen conditions because a mutation unrelated to the checkpoint pathway resulted in decreased *mph1* expression from the *nmt1* promoter. Second, mutant strains may be sensitive to TBZ even though they have an intact spindle checkpoint. For example, mutants in the *S. pombe* tubulin genes, *nda2* and *nda3* (Hiraoka et al., 1984; Toda et al., 1984; Umesono et al., 1983) are sensitive to TBZ because they have defects in microtubule structure. However, *nda2* and *nda3* mutants do initiate a cell cycle delay in response to TBZ treatment, indicating that these mutants have a functional checkpoint pathway.

Previously characterized spindle checkpoint mutants attempt to separate their chromosomes and initiate the next phase of DNA replication when their spindles are damaged (He et al., 1997; Kim et al., 1998). Flow cytometry is a convenient way to determine if cells have initiated the next phase of DNA replication. Therefore, I tested the putative checkpoint mutants from the *mph1* overexpression screen for re-initiation of DNA replication in the presence of disrupted microtubules using three different means of damaging spindle structures: the *cut7* temperature sensitive lethal mutation (Hagan and Yanagida, 1990)and the microtubule destabilizing drugs, TBZ and carbendazim (CBZ).

The *S. pombe cut7* gene encodes a kinesin motor protein which performs an essential function in mitotic spindle formation (Hagan and Yanagida, 1990). Temperature sensitive alleles of *cut7* activate the spindle checkpoint at the restrictive temperature. These cells arrest with an undivided nucleus often "cut" by the septum during cytokinesis. By flow cytometry, it is observed that these cells maintain a 2C DNA content at the restrictive temperature, indicating that the cells have not initiated a new round of DNA replication and that the cells are arrested in the cell cycle. Previously characterized spindle checkpoint deficient strains, such as the *mad2* deletion strain, rapidly re-replicate their DNA in the *cut7* mutant background at the restrictive temperature (Kim et al., 1998).

Double mutants of *cut7* and eight temperature sensitive strains from the *mph1* overexpression screen have been constructed to date. One strain, called 4101, re-initiates DNA replication at the restrictive temperature in the *cut7* mutant background. In contrast, the single 4101 mutant maintains a normal DNA profile at the restrictive temperature (see Figure 2). These results indicate that strain 4101 is unable to block cell cycle progression in the presence of a damaged spindle. Therefore, strain 4101 is defective in the spindle checkpoint.

In addition, strain 4101 is checkpoint deficient when the microtubules are disrupted by two different drugs at the permissive temperature. The microtubule destabilizing drugs, TBZ and CBZ, activate the spindle checkpoint and arrest the cell cycle in wild type cells. These cells arrest at metaphase with 2C DNA content. The 4101 strain has a phenotype similar to the *mad2* deletion strain when treated with TBZ or CBZ. Under conditions in which wild type cells are arrested, 4101 cells and *mad2* deletion cells re-initiate DNA replication (see Figures 3 and 4).

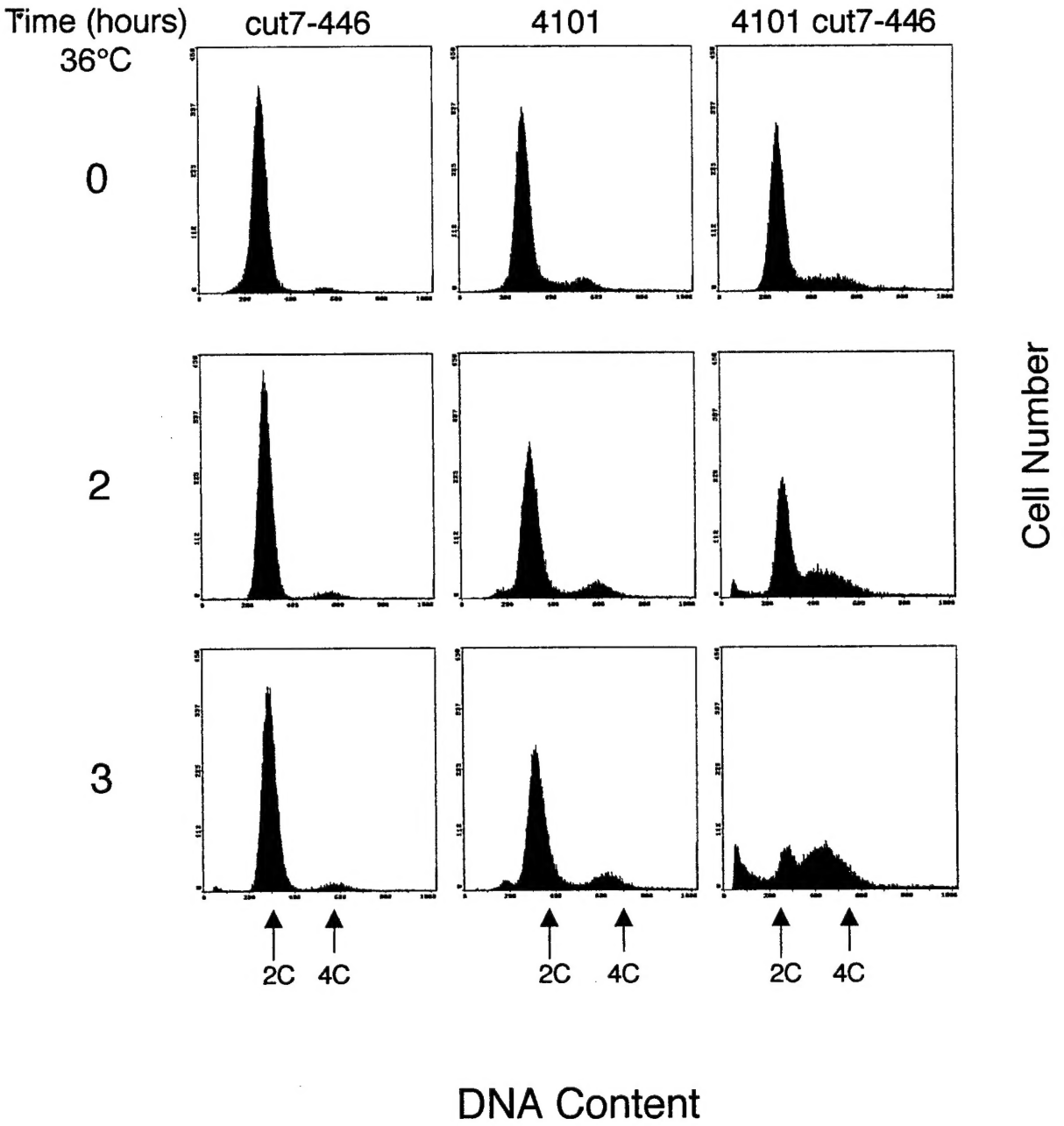

Figure 2: Strain 4101 is defective in the spindle checkpoint. The mutation in strain 4101 was combined with the *cut7-446* temperature sensitive mutation. Cells were shifted to the restrictive temperature, fixed in ethanol at the time points indicated, and processed for flow cytometry. The *cut7* mutation activates the spindle checkpoint at 36°C and initiates a cell cycle arrest with cells maintaining a 2C DNA content. The mutation in strain 4101 abolishes the ability of the *cut7* mutation to activate the spindle checkpoint. Double mutant cells, containing the *cut7* mutation and the *4101* mutation, do not arrest the cell cycle at the restrictive temperature and undergo a second round of DNA replication, as indicated by a large population of cells with greater than 2C DNA content.

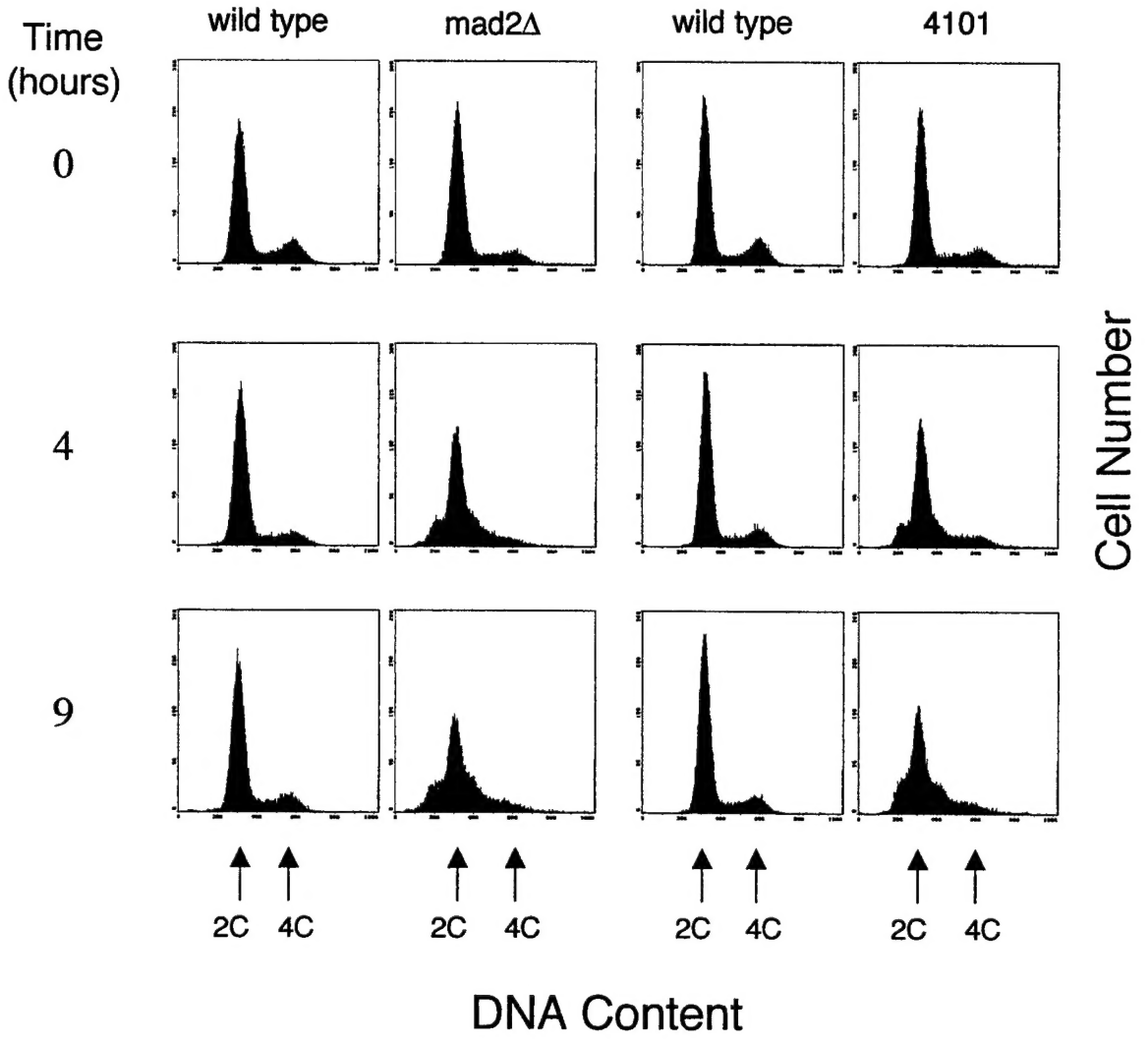

Figure 3: Strain 4101 and the *mad2* deletion strain fail to arrest the cell cycle in response to checkpoint activation by TBZ. Strains were grown at the permissive temperature. At time 0, TBZ was added to a final concentration of 7.5 μg/mL. Cells were fixed in ethanol at the time points indicated and processed for flow cytometry. Under conditions in which the wild type cells remain arrested with 2C DNA content, the 4101 strain and *mad2* deletion strain re-initiate DNA replication as indicated by the presence of cells with DNA content between 2C and 4C.

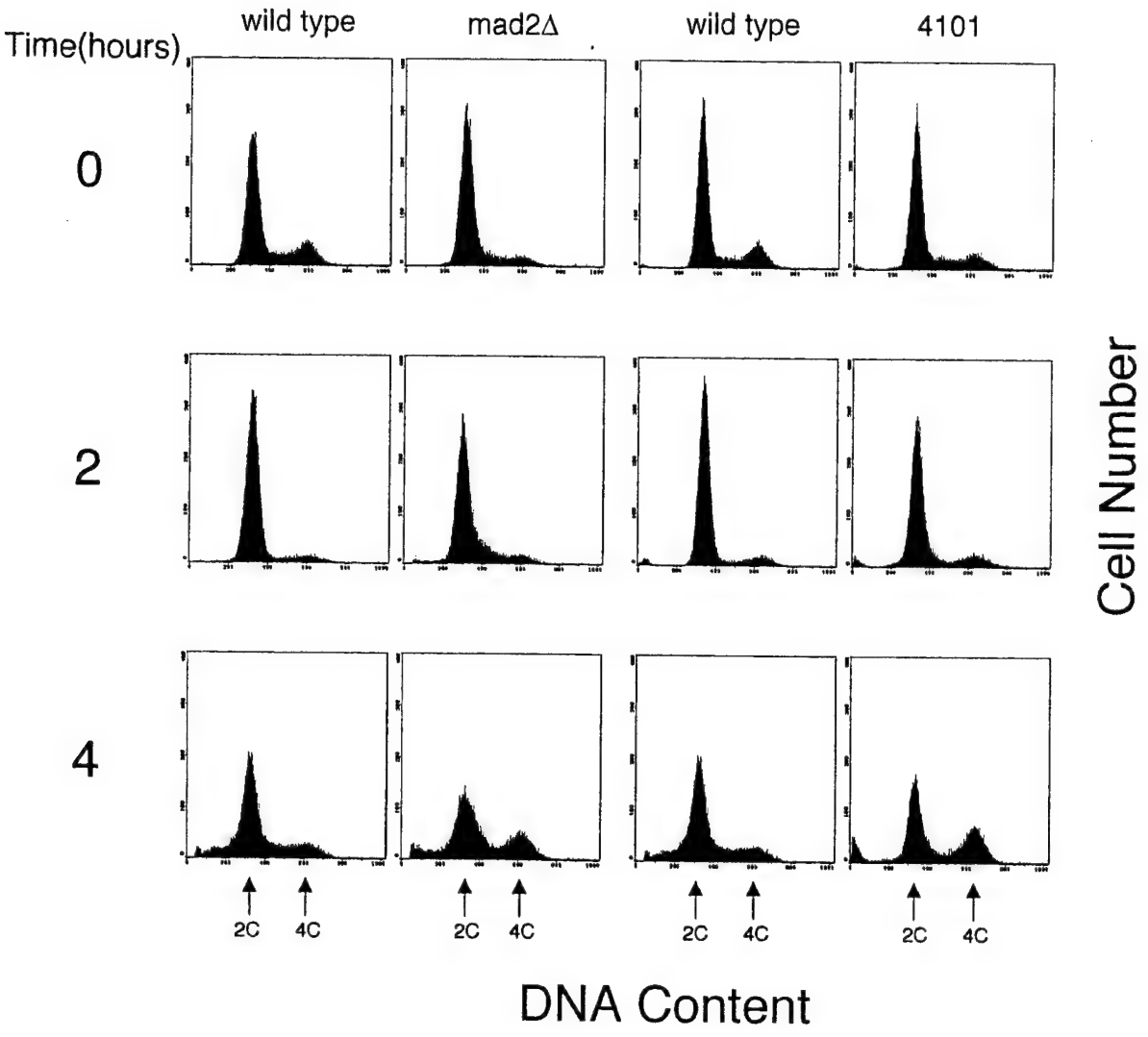

Figure 4: Strain 4101 and the *mad2* deletion strain fail to arrest the cell cycle in response to checkpoint activation by CBZ. Strains were grown at the permissive temperature. At time 0, CBZ was added to a final concentration of 25 μg/mL. Cells were fixed in ethanol at the time points indicated and processed for flow cytometry. Under conditions in which the wild type cells remain arrested with 2C DNA content, the 4101 strain and *mad2* deletion strain re-initiate DNA replication as indicated by the appearance of a population of cells with greater than 2C DNA content.

(3) Strain 4101 encodes a novel component of the spindle checkpoint pathway that acts downstream of *mad2*.

Strain 4101 was previously crossed to strains carrying null mutations of the known spindle checkpoint genes in *S. pombe(mad1, mad2, mad3, bub1*, and *bub3)*, and it was determined that the gene mutated in strain 4101 is distinct from these known spindle checkpoint genes. Strain 4101 was also crossed to the newly identified *S. pombe* spindle checkpoint genes, *alp4* and *alp6* (Vardy and Toda, 2000) and to *slp1*, an essential gene in *S. pombe* that is necessary for cell cycle progression from metaphase to anaphase and is required for the metaphase arrest that follows spindle checkpoint activation (Kim et al., 1998; Matsumoto, 1997). It was observed that the mutation in strain 4101 is not linked to *alp4*, *alp6*, or *slp1*, indicating that strain 4101 is mutated in a novel spindle checkpoint gene.

Next, it was confirmed that the mutation in strain 4101 acts downstream of *mph1* by overexpressing *mph1* from the same *nmt1* regulatable promoter that was used in the *mph1* overexpression screen. Whereas *mph1* overexpression is toxic to wild type cells, strain 4101 cells behave similarly to *mad2* deletion cells since they survive *mph1* overexpression, confirming that strain 4101 is mutated in a gene that acts downstream of *mph1*. In addition, strain 4101 cells can survive overexpression of *mad2*, an indication that the gene mutated in strain 4101 acts downstream of *mad2*.

(4) Strain 4101 cells undergo an abnormal mitosis at the restrictive temperature.

Since the gene mutated in strain 4101 causes temperature sensitive lethality, microscopic analysis of strain 4101 was performed after incubation at the restrictive temperature to observe the terminal phenotype. After 4 hours at 36°C, 4101 cells undergo an abnormal mitosis as evidenced by the following phenotypic observations: chromosome mis-segregation, lagging chromosomes, cell division offset from the center of the cell, and condensed DNA that is stretched across the center of the cell (see Figure 5). These observations are consistent with spindle checkpoint deficiency because proceeding through the cell cycle before kinetochores are properly attached to the mitotic spindle could promote the observed phenotypes.

(5) Identifying the gene mutated in strain 4101

As described in Figure 1, the strategy I have chosen to employ in identifying the mutated gene in selected strains is plasmid rescue of the mutant phenotypes. To date, 23 plasmids have been isolated that rescue the ts phenotype of strain 4101. This collection of plasmids includes 3 genomic plasmids containing genes that are expressed from the native promoter and 20 plasmids containing cDNAs that are expressed from the regulatable *nmt1* promoter (Maundrell, 1993). Current efforts focus on determining if any of these plasmids contain the gene mutated in strain 4101 and sequencing the inserts from the rescuing plasmids to determine the identity of the mutated gene in strain 4101 and other suppressors of the 4101 mutation.

Specific Aim 2: Identify the functional regions of the human Mad2 protein.
No work has been completed on this aim.

Specific Aim 3: Identify new components of the human spindle checkpoint pathway.
No work has been completed on this aim.

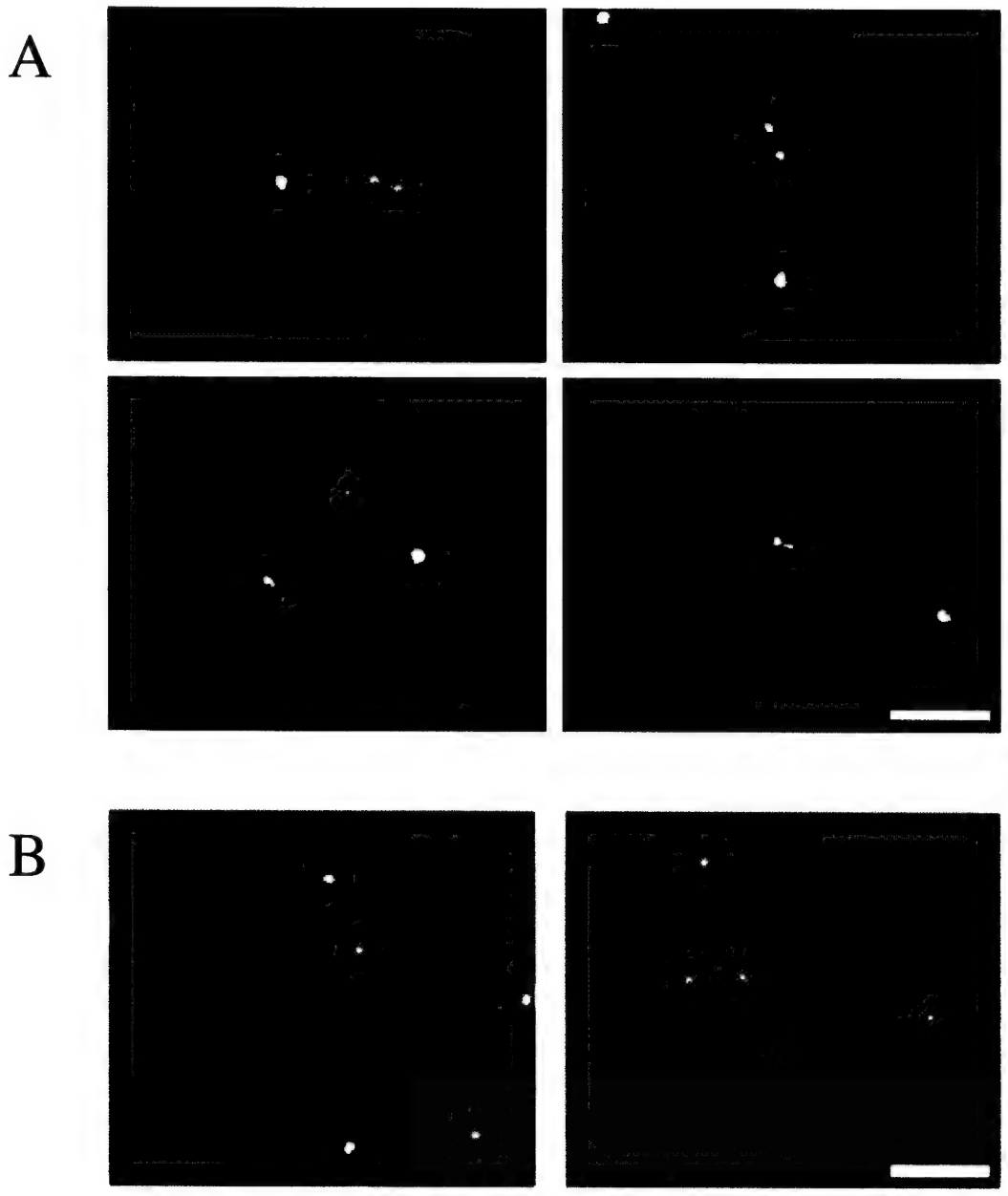

Figure 5: Strain 4101 undergoes an abnormal mitosis at the restrictive temperature. (A) After 4 hours at 36°C, strain 4101 exhibits lagging chromosomes (top left), chromosome mis-segregation (bottom left), cell division offset from the center of the cell (top right), and stretched DNA (bottom right). **(B)** Wild type cells undergo a normal mitosis after 4 hours at 36°C.

KEY RESEARCH ACCOMPLISHMENTS

- The *mph1* overexpression screen has identified three known spindle checkpoint genes: *bub1*, *bub3*, and *mad1*. These results indicate the screen was successful in identifying spindle checkpoint genes and will likely also identify new genes in this pathway.

- The *mph1* overexpression screen has identified one novel component of the spindle checkpoint pathway mutated in a strain called 4101.

- The gene mutated in strain 4101 acts downstream of *mph1* and *mad2*.

- Strain 4101 exhibits mitotic defects at the restrictive temperature that are consistent with deficiency in the spindle checkpoint.

- 23 plasmids have been identified which rescue the temperature sensitivity of strain 4101. Current efforts focus on determining which of these plasmids encode the gene mutated in strain 4101.

REPORTABLE OUTCOMES

The following abstract was submitted to the 2000 FASEB Yeast Chromosome Structure Meeting held in Snowmass Village, Colorado. My abstract was accepted, and I presented a poster at this meeting.

The spindle assembly checkpoint in fission yeast

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During anaphase of mitosis, sister chromatids are separated by the dynamic microtubules which comprise the mitotic spindle. Failure to achieve equal distribution of the chromosomal DNA can lead to genetic loss and lethality. In eukaryotic cells, the spindle assembly checkpoint monitors the structural integrity of the spindle and initiates a cell cycle delay to allow for spindle repair. The fission yeast, *Schizosaccharomyces pombe*, is a useful system for discovering and characterizing components of this regulatory pathway because genetic approaches can be coupled with excellent cytology. In *S. pombe*, spindle checkpoint mutants can be identified by their sensitivity to the microtubule destabilizing drug, thiabendazole (TBZ). Wild type cells arrest the cell cycle in response to the drug and repair spindle damage before anaphase while checkpoint mutants fail to arrest and undergo an aberrant division that results in cell death. Previous work in our lab identified the first two spindle checkpoint genes in *S. pombe* -- *mad2* and *mph1*. Overexpression of either *mad2* or *mph1* activates the spindle checkpoint, arrests cells prior to anaphase, and is toxic to wild type cells. *mph1* acts upstream of *mad2*, and the toxic effect of *mph1* overexpression is suppressed by a *mad2* deletion.

This project aims to discover and characterize novel components of the spindle checkpoint pathway by identifying other suppressors of *mph1* overexpression. Several lines of evidence indicate that this screen has produced spindle checkpoint mutants: the strains are sensitive to TBZ; three of twenty strains tested to date carry mutations in known spindle checkpoint components; and temperature sensitive strains display lagging chromosomes and mis-segregation of DNA at the restrictive temperature. The identification of the gene mutated in selected strains will increase our understanding of the spindle checkpoint by allowing characterization of new components of this pathway.

CONCLUSIONS

The *mph1* overexpression screen has successfully identified three known spindle checkpoint genes and one novel spindle checkpoint gene that is mutated in strain 4101. Strain 4101 cells are checkpoint deficient as indicated by their failure to arrest the cell cycle in response to spindle damage induced by the *cut7-446* mutation, TBZ, or CBZ. Current efforts focus on identifying the novel checkpoint gene that is mutated in strain 4101. At this time, 23 plasmids which rescue the temperature sensitive phenotype of strain 4101 are being tested to determine if they contain the gene mutated in strain 4101 or if they contain high copy suppressors of the 4101 mutation, which would also be informative.

After the gene mutated in strain 4101 is identified, further experiments will be performed to determine how the protein encoded by the new checkpoint gene functions in the checkpoint pathway. This new information will further our understanding of the spindle checkpoint mechanism. In addition, most of the spindle checkpoint genes identified in yeast have mammalian homologues (Cahill et al., 1999; Fisk and Winey, 2001; Jin et al., 1998; Li and Benezra, 1996; Ouyang et al., 1998; Taylor et al., 1998). It has been hypothesized that loss of the spindle checkpoint may promote the multiple genetic changes that mark the development of tumor cells (Cahill et al., 1998; Lengauer et al., 1998; Orr-Weaver and Weinberg, 1998). In support of this prediction, loss of checkpoint function has been observed in many human cancer cell lines (Cahill et al., 1998; Takahashi et al., 1999). Also, decreased expression levels of the checkpoint component, *hsMAD2*, have been demonstrated in human breast cancers (Li and Benezra, 1996) and shown to promote lung tumors in mice (Michel et al., 2001). It is anticipated that the spindle checkpoint gene mutated in strain 4101 will also have a mammalian homologue, and experiments in mammalian systems will be able to determine if mutations in this gene can promote tumorigenesis.

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